The Use of Penicillamine as an Adjuvant to Tartar Emetic in the Treatment of Experimental Schistosomiasis *

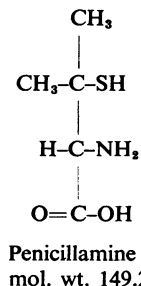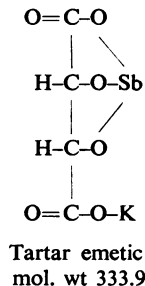M. T. KHAYYAL,[1] N. I. GIRGIS [2] & E. McCONNELL [2]

One of the principal drawbacks of antimonial therapy in schistosomiasis has been the prevalence of annoying, and sometimes dangerous, side-effects. The adjuvant administration of chelating agents offers a possible solution to this problem, providing this can be achieved without appreciably decreasing the therapeutic effect of the drug.

The authors found that the chelating agent penicillamine lowered the toxicity of tartar emetic for mice and hamsters without affecting the tissue uptake of antimony. When administered in a similar manner to hamsters infected with Schistosoma mansoni *there was no effect on the uptake of antimony by the parasites, or on the cure rate. This suggests a potential usefulness of penicillamine in antimony therapy.*

Penicillamine, a sulfur-containing amino-acid, has been shown by many authors to promote the detoxification of various metal ions in the body, probably by virtue of its chelating properties. Walshe (1963, 1964) showed that it enhances copper excretion in patients suffering from Wilson's disease. Boulding & Baker (1957) found it effective in cases of lead poisoning and showed that it also promotes iron excretion. Experimentally, the drug proved of value in protecting rats from lethal doses of mercuric chloride (Aphosian, 1958), but its efficacy in human mercurial intoxication is disputed (Teisinger & Srbova, 1964; Kazantzis et al., 1962; Enneking & Peters, 1964). Eyring & Engleman (1963) found it to be of value in gold poisoning.

In view of the effectiveness of penicillamine in heavy-metal poisoning, it was decided to investigate the effect of this drug on the toxicity (LD_{50}) of tartar emetic for experimental animals. The molecular

formulae and molecular weights of these two compounds are given below.

O=C-O
  | \
H-C-O-Sb
  | /
H-C-O
  |
O=C-O-K

Tartar emetic
mol. wt 333.9

CH₃
 |
CH₃-C-SH
 |
H-C-NH₂
 |
O=C-OH

Penicillamine
mol. wt. 149.2

The effect of penicillamine on the uptake of antinomy by various tissues was also studied to determine whether or not an observed reduction in toxicity could be correlated with a diminished tissue uptake of antimony.

Our findings led us to the simultaneous use of both drugs in the treatment of experimental *Schistosoma mansoni* infections in animals in an effort to determine whether the reduction of antimony toxicity to the host was accompanied by a reduction in the effectiveness of the drug against the parasites. The effects of tartar emetic plus penicillamine on hepatic shift, antimony uptake, and parasite mortality were compared with those of tartar emetic administered alone.

MATERIALS

Penicillamine was obtained through the kindness of Dista Products, Liverpool, England. The ^{124}Sb-labelled tartar emetic was purchased from the Radiochemical Centre, Amersham, England. Radioactive counting was performed using a Nuclear Chicago Scintillation Counter Model 132A.

METHODS

Effect of penicillamine on the LD_{50} of tartar emetic

Mice. Five groups of white albino mice, 10 animals each, were injected intraperitoneally with doses of 30 mg, 40 mg, 50 mg, 60 mg and 70 mg tartar emetic per kg body-weight respectively. The percentage mortality in each group was observed 24 hours after injection and this was plotted against the logarithm of the dose to obtain a control curve for the toxicity of tartar emetic alone.

Another 10 groups, each containing 10 mice from the same strain, were injected intraperitoneally with doses increasing in steps of 10 mg/kg from 50 mg/kg up to 140 mg/kg of tartar emetic, together with twice as much freshly prepared penicillamine solution by the same route. The mortality per group was observed after 24 hours and a toxicity curve plotted.

Hamsters. Four groups of hamsters, of 10 animals each, were injected intraperitoneally with 20 mg, 30 mg, 40 mg and 50 mg of tartar emetic per kg of body-weight, respectively. Three additional groups, of 7 animals each, received 30 mg/kg, 40 mg/kg and 50 mg/kg of tartar emetic in conjunction with twice as much penicillamine as described above. The percentage mortality at 24 hours was determined for each group.

Influence of penicillamine on tissue uptake and excretion of tartar emetic in uninfected hamsters

Two groups of 7 hamsters each were injected intraperitoneally with 8 mg/kg of ^{124}Sb-labelled tartar emetic. One group simultaneously received 18 mg/kg of penicillamine; the other group served as a control. The animals were placed separately in metabolic cages for 24 hours, after which they were sacrificed and samples of liver, blood, heart, urine and faeces from each animal were taken for antimony determination.

Effect of penicillamine on efficacy of tartar emetic administered to hamsters infected with S. mansoni

Hamsters weighing 100 g-150 g each were used 6-7 weeks after exposure to approximately 200 *S. mansoni* cercariae per hamster.

Hepatic shift and antimony uptake. Infected hamsters were divided into 3 groups of 12 animals each. One group received ^{124}Sb-labelled tartar emetic at 8 mg/kg, another group received the same dose of tartar emetic and 18 mg/kg of penicillamine, and the last group served as a control. Injections were given intraperitoneally. The animals were autopsied 24 hours after injection and the worms in the liver and mesenteric veins collected separately by perfusion, separated by sex, and counted. The distribution of female worms, being more indicative of hepatic shift, was tabulated.

Samples of blood, liver and heart were taken from the treated hamsters and the antimony content was derived from their level of radioactivity. The worms from the mesentery and liver of each hamster were pooled as total males and total females before measuring their radioactivity.

Cure rate. Three groups of 7 infected hamsters each were used. One group received 4 mg/kg of tartar emetic per day intraperitoneally for 5 successive days, i.e., 20 mg/kg in all. The second group received daily intraperitoneal injections of 9 mg/kg of penicillamine in addition to the tartar emetic for the same period. The last group received 5 daily injections of penicillamine alone at 9 mg/kg/day. The animals were sacrified 10 days after the last dose and the worms in the mesenteric veins and liver were perfused out separately. Ensheathed worms were not recovered by perfusion but were counted by the liver-crush technique. The assessment of the cure rate was made on the basis of dead worms in the liver expressed as a percentage of the total worm burden.

RESULTS

The effect of penicillamine in reducing the acute toxicity of tartar emetic to mice and hamsters is presented in the accompanying figure and in Table 1, respectively. In mice, the simultaneous administration of penicillamine led to a nearly twofold increase in the LD_{50} of tartar emetic, from approximately 50 mg/kg to about 90 mg/kg. The toxicity was not diminished further by using higher doses of penicillamine. Essentially similar results were observed in hamsters, where the LD_{50} of tartar emetic was found to be about 35 mg/kg and the LD_{100} approximately 50 mg/kg. At these dose levels the concurrent administration of penicillamine reduced the mortality to zero.

Table 2 compares the tissue levels of antimony as well as total antimony excretion by normal

EFFECT OF SIMULTANEOUS ADMINISTRATION OF PENICILLAMINE
ON THE MORTALITY CURVE FOR TARTAR EMETIC IN MICE [a]
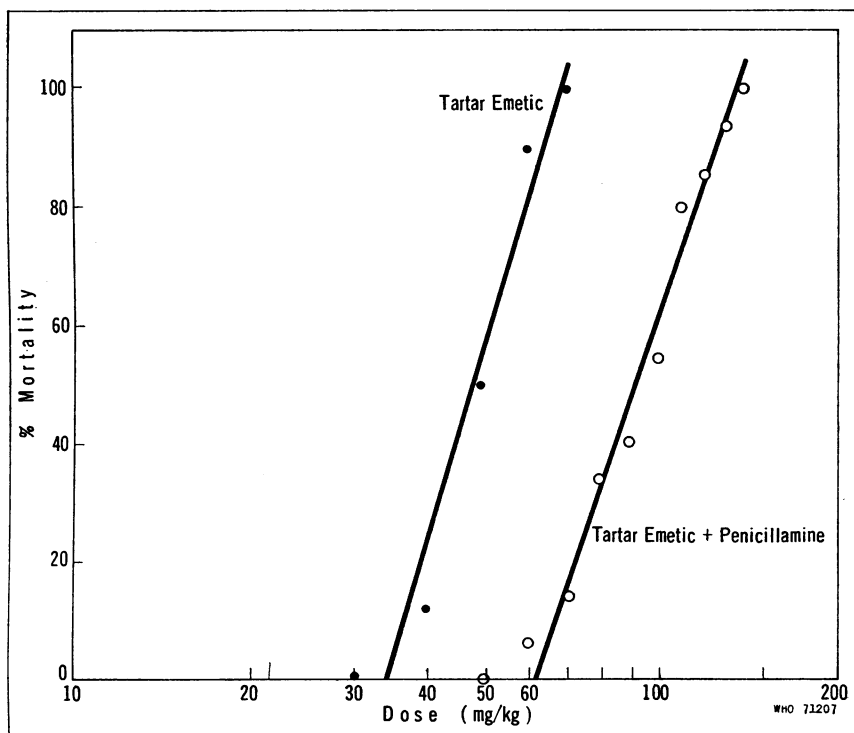
[a] The doses of penicillamine used were twice those of tartar emetic.

hamsters 24 hours after administration of tartar emetic alone and in conjunction with penicillamine.

It is evident that there was no significant difference in tissue antimony levels or excretion rates between the two groups. A comparison of antimony uptake

TABLE 1
EFFECTS OF PENICILLAMINE
ON THE TOXICITY OF TARTAR EMETIC IN HAMSTERS

Tartar emetic (mg/kg)	Mortality (%)	
	Tartar emetic alone	Tartar emetic plus penicillamine[a]
20	0	—
30	20	0
40	80	0
50	100	0

[a] The doses of penicillamine used were twice those of tartar emetic.

by the tissues of normal and infected hamsters (Tables 2 and 4) shows a slightly higher level in the blood and heart of both groups of infected animals (i.e., those with and without penicillamine), but no apparent difference in the liver content.

The effect of the drugs tested on the relative distribution of female worms in the infected hamsters 24 hours after injection is shown in Table 3. There was no significant difference between the hepatic shift produced when tartar emetic was administered alone or with penicillamine. In both cases the percentage of female worms found in the liver was 5-6 times that of the controls.

Table 4 shows the antimony levels in the blood, liver, and heart of the above hamsters as well as in the male and female schistosomes obtained from them. The results are expressed in terms of μg of antimony per g of tissue or per g of schistosomes. Student's t-test showed no significant differences between the mean values obtained for the tissue and worm antimony levels in the two groups.

TABLE 2

DISTRIBUTION OF ANTIMONY IN NORMAL HAMSTERS 24 HOURS
AFTER INJECTION OF TARTAR EMETIC (8 mg/kg)
WITH AND WITHOUT PENICILLAMINE (18 mg/kg) [a]

| | Antimony concentration (μg/g) | | | Percentage of dose excreted |
	Blood	Liver	Heart	
Tartar emetic alone	0.16 ± 0.02	14.20 ± 2.19	0.16 ± 0.02	42.60 ± 5.20
Tartar emetic plus penicillamine	0.17 ± 0.01	16.48 ± 3.39	0.15 ± 0.01	42.38 ± 4.46

[a] All results are given as mean ± standard error.

TABLE 3

DISTRIBUTION OF FEMALE WORMS IN INFECTED HAMSTERS 24 HOURS
AFTER A SINGLE DOSE OF TARTAR EMETIC (8 mg/kg)
WITH AND WITHOUT PENICILLAMINE (18 mg/kg) [a]

	No. of worms per hamster	Percentage of female worms	Percentage of female worms in liver
Tartar emetic	81.54 ± 10.11	34.24 ± 3.57	29.80 ± 4.44
Tartar emetic plus penicillamine	82.45 ± 11.72	36.48 ± 3.35	25.54 ± 5.34
Control	70.20 ± 3.42	29.37 ± 2.41	5.05 ± 1.05

[a] All results are given as mean ± standard error.

TABLE 4

ANTIMONY LEVELS IN TISSUES AND SCHISTOSOMES FROM INFECTED HAMSTERS 24 HOURS AFTER
ADMINISTRATION OF TARTAR EMETIC (8 mg/kg) WITH AND WITHOUT PENICILLAMINE (18 mg/kg) [a]

| | Antimony concentration (μg/g) | | | | |
	Blood	Liver	Heart	Male worms	Female worms
Tartar emetic	0.24 ± 0.03	14.72 ± 0.87	0.27 ± 0.02	17.09 ± 2.51	86.87 ± 4.95
Tartar emetic plus penicillamine	0.21 ± 0.02	17.90 ± 1.81	0.33 ± 0.05	14.45 ± 1.02	79.80 ± 5.43

[a] All results are given as mean ± standard error.

TABLE 5

WORM MORTALITY IN INFECTED HAMSTERS 10 DAYS
AFTER FINAL INJECTION OF TARTAR EMETIC
(4 mg/kg/DAY FOR 5 DAYS) WITH AND WITHOUT
PENICILLAMINE (9 mg/kg/DAY) [a]

	No. of worms per hamster	Percentage dead worms in liver
Tartar emetic	53.66 ± 8.34	83.23 ± 3.68
Tartar emetic plus penicillamine	51.66 ± 10.91	84.73 ± 4.93

[a] All results are given as mean ± standard error.

The cure rates in infected hamsters receiving tartar emetic either alone or with penicillamine are shown in Table 5. No significant difference was found between the two groups. Penicillamine alone produced no parasite mortality.

DISCUSSION

It can be seen from the above findings that in experimental animals penicillamine affords a degree of protection against the acute lethal effects of tartar emetic. This result is in agreement with that arrived

at by other workers investigating the efficacy of penicillamine in various heavy-metal intoxications.

The mechanism of action of this drug probably depends on chelation of the metallic ions by its −SH groups. These metal chelates are in some cases more amenable to excretion.

The exact mechanism of the chelation process, however, may vary from one metal to another, as shown by Doornbos & Faber (1964) who found that whereas zinc, for example, is bound to sulfur and nitrogen, lead and mercury are not. The mode of chelation of antimony has yet to be resolved.

The clinical toxicity of tartar emetic resides mainly in its toxicity to the heart and liver, and hence it was of interest to see whether the diminished toxicity of the drug when given with penicillamine was correlated with decreased antimony uptake by these tissues, or with an increased excretion rate. However, our results showed no significant difference between the test group (tartar emetic plus penicillamine) and the control group (tartar emetic only) of animals for either tissue uptake or excretion of antimony. Accordingly, the reduction in toxicity cannot be explained by a simple increase in the excretion of the compound, or a diminished uptake. A more elaborate mechanism is probably involved. Although the total trivalent-antimony levels in both test and control groups are nearly the same, the chemical form in which the antimony is present is probably different. The " chelated " compound, if chelation is the process involved, is possibly better tolerated by the host than the free antimonial.

Single doses of tartar emetic are known to induce temporary paralysis of the schistosomes in the mesenteric veins of infected animals, resulting in the worms being swept into the liver—the effect known as hepatic shift (Buttle & Khayyal, 1962). The character and magnitude of the shift is related to the antimony level in the worms (Khayyal, 1964), which varies with the dose of the drug (Khayyal, 1965a). The shift of female worms was used as an index of drug activity in the present study in preference to the total worm shift. Female worms were nearly all found paired in the mesenteric veins of the control animals and it was felt that their translocation would give a truer picture of drug action (Standen, 1950). The shift of female worms observed 24 hours after drug administration was approximately the same with either emetic alone or tartar emetic plus penicillamine, there being no significant difference between the shift values for the two groups. Further evidence to this effect was provided by the fact that penicil-

lamine did not influence the uptake of antimony by the parasites nor by the host tissues studied— the blood, liver and heart (Table 5). The lack of effect of the drug on worm antimony levels is in agreement with the unchanged hepatic shift, since the two effects are interdependent. Similarly, the lack of effect on tissue antimony uptake is consistent with our findings (Table 2) that penicillamine does not affect the level of antimony in the liver and heart. It seems probable that the penicillamine-tartar-emetic complex is capable of gaining access to the host tissues and to the parasites with as much ease as tartar emetic alone.

Although not specifically related to the current study, the higher antimony levels in the blood and heart of infected hamsters are of interest. Whether or not this observation is of special significance remains to be determined.

Just as the worm shift and the worm antimony levels were not significantly altered by using penicillamine with tartar emetic, neither were the cure rates. This is in agreement with the view that the cure rate depends upon the effective antimony concentration within the worms, which remains unaltered whether or not penicillamine is given with the tartar emetic.

It was shown above that penicillamine lowers the acute toxicity of tartar emetic for hamsters and white mice though it does not affect the host tissue-antimony levels. This effect was attributed to the probable formation of an antimony–penicillamine chelated complex which is less toxic to the host. Is seems from the present findings that this complex is as toxic to the schistosomes as the tartar emetic alone. Similar results had been obtained earlier using dimercaprol, another chelating agent (Khayyal, 1965b) but the latter is much more toxic to the host than penicillamine.

The therapeutic usefulness of antimonials probably depends on a difference in the mechanism of toxicity towards host and parasite. Thus, whereas antimonials inhibit worm phosphofructokinase (Bueding & Mansour, 1957) and interfere with glycolysis which represents the main source of energy for the worms (Bueding, 1950; Bueding & Peters, 1951), the mammalian toxicity is probably related to an inhibition of sulfhydryl enzymes. Mammalian phosphofructokinase has been shown to be much less sensitive to the inhibitory effect of the antimonials (Mansour & Bueding, 1954). It should thus be possible to inhibit one system preferentially without affecting the other.

Penicillamine, being a thiol compound like dimercaprol, tends to protect the host against the harmful effects of antimony, and has the advantage of being less toxic. The antimony–penicillamine complex, although having less toxicity for the host, does not seem to affect the ability of antimony to inhibit schistosome metabolism. Penicillamine has thus proved of value in experimental schistosomiasis and may be useful in clinical cases for alleviating the side-effects of the antimony preparations which are, perhaps unfortunately, still the drugs of choice.

RÉSUMÉ

La pénicillamine, aminoacide soufré, facilite la détoxication des ions métalliques introduits dans l'organisme, vraisemblablement grâce à son action chélatrice. On a recherché chez l'animal si cette propriété pouvait être mise à profit pour atténuer l'effet toxique de l'administration de tartre stibié.

Cinquante souris témoins ont reçu par voie intrapéritonéale 30 à 70 mg par kg de poids corporel de tartre stibié et 100 autres ont été traitées par 50 à 140 mg/kg du même produit associés à une dose double de pénicillamine. Une expérience similaire a été menée sur deux groupes de hamsters. Chez les deux espèces animales, l'administration de pénicillamine a eu pour résultat de diminuer fortement la toxicité aiguë du tartre stibié, la DL$_{50}$ passant chez les souris de 50 à 90 mg/kg environ.

Deux groupes de hamsters ayant reçu, l'un 8 mg/kg de tartre stibié marqué à l'antimoine-124 (groupe témoin), le second une dose identique de ce composé et 18 mg/kg de pénicillamine ont été sacrifiés après 24 heures. Aucune différence significative n'a été observée entre les deux groupes concernant les taux d'antimoine existant dans les tissus (foie, cœur, sang) et les quantités du métal excrétées par l'urine ou les matières fécales.

On a ensuite recherché l'effet de l'injection de pénicillamine au cours du traitement par le tartre stibié de hamsters infectés expérimentalement par *Schistosoma mansoni*. A l'autopsie, pratiquée après 24 heures, d'animaux traités soit par le tartre stibié seul soit par l'association tartre stibié-pénicillamine, on n'a noté aucune différence significative entre les nombres de schistosomes présents dans le foie des animaux des deux groupes. Chez tous les hamsters traités, les pourcentages de parasites femelles découverts dans le foie étaient cinq à six fois plus élevés que chez des hamsters témoins infectés mais non traités. Les teneurs en antimoine du foie, du cœur, du sang et des parasites étaient très voisines chez tous les animaux traités, qu'ils aient reçu ou non de la pénicillamine.

Enfin, trois groupes de hamsters infectés ont été traités, le premier par le tartre stibié seul, le deuxième par l'association tartre stibié-pénicillamine, le troisième par la pénicillamine seule, et sacrifiés après 10 jours. Le taux de guérison, exprimé par le pourcentage de parasites morts trouvés dans le foie par rapport à la charge parasitaire totale, a été très voisin dans les deux premiers groupes. L'administration de pénicillamine seule n'a entraîné aucune mortalité des schistosomes.

Les auteurs discutent les mécanismes par lesquels la pénicillamine réduit la toxicité du composé antimonié sans altérer sa captation par les tissus.

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